JC10 Rec'd PCT/PTO 22 JAN 2002

FORM PTO-1390 (REV. 12-2001) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: TJK/209

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 10/031938

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/06870	July 18, 2000	July 21, 1999

TITLE OF INVENTION: CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES

APPLICANT(S) FOR DO/EO/US: Srinivas Kaveri et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [X] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information: *Check in the amount of $1,976.00
    *Certificate of Express Mail
    *Return post card

JC13 Rec'd PCT/PTO 22 JAN 2002

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/051938	PCT/EP00/06870	TJK/209

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		CALCULATIONS PTO USE ONLY	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =		$890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	57 - 20 =	37	37 x $18.00	$ 666.00	
Independent claims	8 - 3 =	5	5 x $84.00	$ 420.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$0	
TOTAL OF ABOVE CALCULATIONS =				$ 1,976.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$	
SUBTOTAL =				$ 1,976.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ 0	
TOTAL NATIONAL FEE =				$1,976.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 1,976.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 1,976.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-2126. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Timothy J. Keefer
Wildman, Harrold, Allen & Dixon
225 West Wacker Drive
Chicago, Illinois 60606

SIGNATURE

Timothy J. Keefer
NAME

35,567
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Kaveri et al.	)	CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES
Serial No.: 10/031,938	)	
Filed: January 22, 2002	)	Attorney Docket: TJK/209
	)	Group Art Unit:
	)	Examiner:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on Jan 31, 2003.

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Date of Signature: Jan 31, 2003

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT OF SEQUENCE LISTING

Please cancel the sequence listing currently on file and replace it with attached sequence listing. Substitute sheets containing the amended sequence listing are enclosed per 37 CFR § 1.825(a). The sequence listing has been amended to conform to the requirements if 37 CFR §§ 1.821-1.823, in response to the Notice Of Defective Response mailed on December 31, 2002. The substitute sheets contain no new matter. A diskette containing a copy of the amended

sequence listing is enclosed per 37 CFR 1.825(b). the sequence listing on the diskette is the same as the substitute paper copy submitted with the amendment.

Applicant notes that on previous attempts to submit a copy of the sequence listing on a computer readable medium, the medium has become corrupted, thereby rendering the sequence listing unreadable. Applicant respectfully submits that he should not be penalized for untimely submission of the sequence listing when the media is corrupted during transport, and that steps will be taken to hand-carry a computer-readable copy of the sequence listing into the USPTO if the diskettes attached hereto become corrupted.

Respectfully submitted,

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Dated: 31 January, 2003

WILDMAN, HARROLD, ALLEN & DIXON
225 West Wacker Drive
Chicago, Illinois 60606-1229
Telephone: (312) 201-2834
Facsimile: (312) 201-2327
e-mail: keefer@wildmanharrold.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of :
Srinivas KAVERI *et al.*
Serial No. :Not yet known
(PCT/EP00/06870 of 18.07.00)
Filed : Concurrently herewith

Group Art Unit :
Examiner :

For :**CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES.**

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Honorable Sir,

Before calculation of the filing fee, please amend the above-identified patent application as follows :

IN THE CLAIMS:

Please cancel original claims 1 to 27, and add the following claims 28 to 85, without prejudice or disclaimer of the subject matter thereof.

CLAIMS

28. A method of determining the presence of anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, which comprises :

i) isolating the plasma from a sample of blood taken from said mammal,

ii) isolating anti-Factor VIII allo-antibodies from said plasma ;

iii) placing said anti-Factor VIII allo-antibodies in contact with Factor VIII for a period of time sufficient to permit any degradation of said Factor VIII by said anti-Factor VIII allo-antibodies ; and

iv) determining, after said period of time, whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies.

29. The method of claim 28, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated from said plasma by combining them with said Factor VIII.

30. The method of claim 29, wherein said Factor VIII is coupled to a matrix.

31. The method of claim 28, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated by affinity chromatography.

32. The method of claim 31, wherein in step ii), said affinity chromatography comprises the use of Factor VIII covalently coupled to a Sepharose matrix.

33. The method of claim 32, wherein said Sepharose matrix is activated with cyanogen bromide.

34. The method of claim 28, wherein in step iii), said Factor VIII is labelled with a labelling agent.

35. The method of claim 34, wherein said labelling agent is a radio-labelling agent.

36. The method of claim 35, wherein said radio-labelling agent is ^{125}I.

37. The method of claim 28, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of about 15 to about 40°C.

38. The method of claim 28, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of about 15 to about 40°C.

39. The method of claim 28, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of 38°C.

40. The method of claim 28, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of 38°C.

41. The method of claim 28, wherein step iv) is carried out by a determination comprising a separation technique and a visualisation technique.

42. The method of claim 41, wherein said separation technique is selected from the group consisting of gel electrophoresis, and gel filtration.

43. The method of claim 42, wherein said gel electrophoresis is SDS PAGE.

44. The method of claim 42, wherein said gel filtration is fast protein liquid chromatography gel filtration.

45. The method of claim 42, wherein said visualisation technique is autoradiography.

46. The method of claim 28, which further comprises :

v) characterising the site(s) in said Factor VIII molecule cleaved by said anti-Factor VIII allo-antibodies.

47. The method of claim 46, wherein said characterisation is carried out by placing said Factor VIII in contact with said anti-Factor VIII allo-antibodies capable of degrading Factor VIII, separating and then sequencing the fragments of Factor VIII resulting therefrom.

48. The method of claim 47, wherein said separation is carried out using a technique such as gel electrophoresis.

49. The method of claim 48, wherein said separation is SDS PAGE.

50. The method of claim 47, wherein said sequencing is carried out using a technique such as N-terminal sequencing.

51. The method of claim 50, wherein said sequencing carried out using a technique such as N-terminal sequencing is by using an automatic protein microsequencer.

52. The method of claim 46, wherein said sequencing locates scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

53. An amino acid sequence :

Ser Val Ala Lys Lys His Pro .

54. An amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

55. An amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

56. A peptide or non-peptide analogue of an amino acid sequence of claim 53, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

57. A peptide or non-peptide analogue of an amino acid sequence of claim 54, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

58. A peptide or non-peptide analogue of an amino acid sequence of claim 55, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

59. An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

60. The inhibitor of claim 59, which comprises a protease inhibitor.

61. The inhibitor of claim 60, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

62. The inhibitor of claim 59, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

63. The inhibitor of claim 59, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

64. The inhibitor of claim 59, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

65. The inhibitor of claim 59, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

66. A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of an anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

67. The pharmaceutical composition of claim 66, wherein said anti-Factor VIII allo-antibody capable of degrading Factor VIII is as obtainable from the method of claim 28.

68. A method of therapeutic treatment of a mammal suffering from a pathology resulting from abnormal level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a

pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier, is administered to said mammal.

69. The method of claim 68, wherein said pathology results from the presence of an excess of Factor VIII in the blood thereof.

70. The method of claim 69, which is a therapeutic treatment of a mammal suffering from thrombosis.

71. A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of a Factor VIII degradation inhibitor of claim 59, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

72. The pharmaceutical composition of claim 71, which comprises a protease inhibitor.

73. The pharmaceutical composition of claim 72, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

74. The pharmaceutical composition of claim 71, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

75. The pharmaceutical composition of claim 71, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

76. The pharmaceutical composition of claim 71, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

77. The pharmaceutical composition of claim 71, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

78. A method of therapeutic treatment of a mammal suffering from a pathology resulting from the sub-physiological level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one Factor VIII degradation inhibitor, and a pharmaceutically acceptable salt thereof, is administered to said mammal.

79. The method of claim 78, wherein said inhibitor comprises a protease inhibitor.

80. The method of claim 79, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

81. The method of claim 78, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

82. The method of claim 78, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

83. The method of claim 78, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

84. The method of claim 78, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

85. The method of claim 78, which is a method of therapeutic treatment of a mammal suffering from haemophilia A.

PTO

REMARKS

This preliminary amendment is filed in order to bring the claims into conformity with US practice and especially to eliminate inappropriate multiple dependency in the claims in this National Phase application based on PCT Application No. PCT/EP00/06870 of 18.07.00.

In view of the foregoing amendments and remarks, Applicants submit that the present application is now in condition for allowance. An early allowance of the application with amended claims is earnestly solicited.

Respectfully submitted,

Registration No. 35,587

CERTIFICATE OF EXPRESS MAIL

"Express Mail" mailing label number EL881436912US

Date of Deposit January 22, 2002

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

Melissa Broadway

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Kaveri et al.	)	CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES
Serial No.: 10/031,938	)	
Filed: January 22, 2002	)	Attorney Docket: TJK/209
	)	Group Art Unit:
	)	Examiner:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on Jan 31, 2003.

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Date of Signature: Jan 31, 2003

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT OF SEQUENCE LISTING

Please cancel the sequence listing currently on file and replace it with attached sequence listing. Substitute sheets containing the amended sequence listing are enclosed per 37 CFR § 1.825(a). The sequence listing has been amended to conform to the requirements if 37 CFR §§ 1.821-1.823, in response to the Notice Of Defective Response mailed on December 31, 2002. The substitute sheets contain no new matter. A diskette containing a copy of the amended

sequence listing is enclosed per 37 CFR 1.825(b). the sequence listing on the diskette is the same as the substitute paper copy submitted with the amendment.

Applicant notes that on previous attempts to submit a copy of the sequence listing on a computer readable medium, the medium has become corrupted, thereby rendering the sequence listing unreadable. Applicant respectfully submits that he should not be penalized for untimely submission of the sequence listing when the media is corrupted during transport, and that steps will be taken to hand-carry a computer-readable copy of the sequence listing into the USPTO if the diskettes attached hereto become corrupted.

Respectfully submitted,

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Dated: Jan 31, 2003

WILDMAN, HARROLD, ALLEN & DIXON
225 West Wacker Drive
Chicago, Illinois 60606-1229
Telephone: (312) 201-2834
Facsimile: (312) 201-2327
e-mail: keefer@wildmanharrold.com

SEQUENCE LISTING

<110> KAVERI, Srinivas

LACROIX-DESMAZES, Sebastien

KAZATCHKINE, Michel

<120> Catalytic anti-factor VIII allo-antibodies

<130> H15303-4US

<140> 10/031,938

<141> 2002-09-12

<150> PCT/EP00/06870

<151> 2000-07-18

<150> EP99401841.4

<151> 1999-07-21

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<400> 2

[redacted]

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<211> 7

<212> PRT

<213> Homo sapiens

<400> 3

Asp Gln Arg Gln Gly Ala Glu
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COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Kaveri et al.

Atty. Docket No. TJK/209

Serial No. 10/031,938

Group Art Unit:

Filed: January 22, 2002

Examiner:

Title: CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

TRANSMITTAL LETTER

In response to the Notice of Defective Response Action dated December 31, 2002, enclosed are the following:

1. Second Preliminary Amendment;

2. Computer Disk; and

3. Check for $618.00 for additional claims which were not initially paid for with the initial filing (58 total claims originally paid for, 8 independent claims originally paid for – Fee for 11 additional claims @ $18.00 = $198.00 - Fee for 5 additional Independent claims @ $84.00 = $420.00);

4. Duplicate Copy of Notification of Defective Response; and

5. Stamped, self-addressed receipt postcard.

Please charge any additional fees which include extension fees if any to Deposit Account No. 23-2126. An extra copy is enclosed.

Date: 1/28/03

Respectfully submitted,

Timothy J. Keefer
Registration No. 35,567

CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on 1/28/2003

Mindy L. Fitch

Mindy L. Fitch

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Kaveri et al.	)	CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES
Serial No.: 10/031,938	)	
Filed: January 22, 2002	)	Attorney Docket: TJK/209
	)	Group Art Unit:
	)	Examiner:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on 1/28, 2003.

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Date of Signature: 1/28, 2003

Assistant Commissioner for Patents
Washington, D.C. 20231

SECOND PRELIMINARY AMENDMENT

IN THE SPECIFICATION:

On page 6, lines 17-32, continuing through to page 7, lines 1-10, please delete the existing paragraph and replace it with the following paragraph:

Hence, according to a third aspect, the present invention provides an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Advantageously, this inhibitor is

characterized in that it comprises a protease inhibitor. Examples of protease inhibitors that can be used as anti-Factor VIII allo-antibody catalysed Factor VIII degradation inhibitors within the context of the present invention, without being limited thereto, are fluorophosphate-type inhibitors, such as DFP for example, or sulphonyl fluoride-type inhibitors, such as PMSF or AEBSF (4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (notably marked by Roche Diagnostics GmbH, Mannheim, Germany, under the trademark Pefabloc®)), for example. More particularly, this inhibitor is characterized in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and the $Glu^{1794} - Asp^{1795}$ located within the A3 domain of the Factor VIII molecule. More preferably still, this inhibitor is characterized in that it comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro ;

a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; or

a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

On page 20, please delete the existing table and replace it with the following table:

Amino acid sequence	Cleavage site
Ser Val Ala Lys Lys His Pro (SVAKKHP)	$Arg^{372} - Ser^{373}$ $(R^{372} - S^{373})$
Asp Gln Arg Gln Gly Ala Glu (DQRQGAE)	$Glu^{1794} - Asp^{1795}$ $(E^{1794} - D^{1795})$
Asp Glu Asp Glu Asn Gln Ser (DEDENQS)	$Tyr^{1680} - Asp^{1681}$ $(Y^{1680} - D^{1681}$

IN THE CLAIMS:

Please cancel pending claims 28 to 85.

Please enter new claims 86 to 154.

86. (New) A method of determining the presence of anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, which comprises :

i) isolating the plasma from a sample of blood taken from said mammal,

ii) isolating anti-Factor VIII allo-antibodies from said plasma ;

iii) placing said anti-Factor VIII allo-antibodies in contact with Factor VIII for a period of time sufficient to permit any degradation of said Factor VIII by said anti-Factor VIII allo-antibodies ; and

iv) determining, after said period of time, whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies.

87. (New) The method of claim 86, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated from said plasma by combining them with said Factor VIII.

88. (New) The method of claim 87, wherein said Factor VIII is coupled to a matrix.

89. (New) The method of claim 86, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated by affinity chromatography.

90. (New) The method of claim 89, wherein in step ii), said affinity chromatography comprises the use of Factor VIII covalently coupled to a Sepharose matrix.

91. (New) The method of claim 90, wherein said Sepharose matrix is activated with cyanogen bromide.

92. (New) The method of claim 86, wherein in step iii), said Factor VIII is labelled with a labelling agent.

93. (New) The method of claim 92, wherein said labelling agent is a radio-labelling agent.

94. (New) The method of claim 93, wherein said radio-labelling agent is ^{125}I.

95. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of about 15 to about 40°C.

96. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of about 15 to about 40°C.

97. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of 38°C.

98. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of 38°C.

99. (New) The method of claim 86, wherein step iv) is carried out by a determination comprising a separation technique and a visualisation technique.

100. (New) The method of claim 99, wherein said separation technique is selected from the group consisting of gel electrophoresis, and gel filtration.

101. (New) The method of claim 100, wherein said gel electrophoresis is SDS PAGE.

102. (New) The method of claim 100, wherein said gel filtration is fast protein liquid chromatography gel filtration.

103. (New) The method of claim 100, wherein said visualisation technique is autoradiography.

104. (New) The method of claim 86, which further comprises :

v) characterising the site(s) in said Factor VIII molecule cleaved by said anti-Factor VIII allo-antibodies.

105. (New) The method of claim 104, wherein said characterisation is carried out by placing said Factor VIII in contact with said anti-Factor VIII allo-antibodies capable of degrading Factor VIII, separating and then sequencing the fragments of Factor VIII resulting therefrom.

106. (New) The method of claim 105, wherein said separation is carried out using a technique such as gel electrophoresis.

107. (New) The method of claim 106, wherein said separation is SDS PAGE.

108. (New) The method of claim 105, wherein said sequencing is carried out using a technique such as N-terminal sequencing.

109. (New) The method of claim 108, wherein said sequencing carried out using a technique such as N-terminal sequencing is by using an automatic protein microsequencer.

110. (New) The method of claim 104, wherein said sequencing locates scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

111. (New) An amino acid sequence :

Ser Val Ala Lys Lys His Pro .

112. (New) An amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

113. (New) An amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

114. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 111, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

115. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 112, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

116. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 113, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

117. (New) A method of neutralising catalytic anti-Factor VIII allo-antibodies comprising using an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

118. (New) The method of claim 117, wherein said inhibitor comprises a protease inhibitor.

119. (New) The method of claim 118, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

[redacted]

120. (New) The method of claim 117, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

121. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

122. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

123. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

124. (New) A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of an anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

125. (New) The pharmaceutical composition of claim 124, wherein said anti-Factor VIII allo-antibody capable of degrading Factor VIII is as obtainable from the method of claim 86.

126. (New) A method of therapeutic treatment of a mammal suffering from a pathology resulting from abnormal level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a

pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier, is administered to said mammal.

127. (New) The method of claim 126, wherein said pathology results from the presence of an excess of Factor VIII in the blood thereof.

128. (New) The method of claim 127, wherein said pathology is of thrombotic nature.

129. (New) The method of claim 128, which is a therapeutic treatment of a mammal suffering from thrombosis.

130. (New) A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of a Factor VIII degradation inhibitor of claim 117, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

131. (New) A method of therapeutic treatment of a mammal suffering from a pathology resulting from the sub-physiological level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one Factor VIII degradation inhibitor, and a pharmaceutically acceptable salt thereof, is administered to said mammal.

132. (New) The method of claim 131, wherein said inhibitor comprises a protease inhibitor.

133. (New) The method of claim 132, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

134. (New) The method of claim 131, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

[redacted]

135. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

136. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

137. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

138. (New) The method of claim 131, wherein said pathology is of haemophilic nature.

139. (New) The method of claim 138, wherein said pathology of haemophilic nature is a disease involving coagulation defects due to Factor VIII insufficiency.

140. (New) The method of claim 138, which is a method of therapeutic treatment of a mammal suffering from haemophilia A.

141. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro .

142. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence:

Asp Glu Asp Glu Asn Gln Ser .

143. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

144. (New) A pharmaceutical composition, which comprises a pharmaceutically effective amount of an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

145. (New) The pharmaceutical composition of claim 144, which comprises a protease inhibitor.

146. (New) The pharmaceutical composition of claim 145, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

147. (New) The pharmaceutical composition of claim 144, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

148. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

[redacted]

149. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

150. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

151. (New) An anti-Factor VIII allo-antibody, which has a catalytic activity capable of catalysing degradation of Factor VIII.

152. (New) An anti-Factor VIII allo-antibody which is obtainable by the method of claim 86.

153. (New) The anti-Factor VIII allo-antibody of claim 151, which cleaves the following scissile bonds in the Factor VIII molecule: Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

154. (New) The anti-Factor VIII allo-antibody of claim 152, which cleaves the following scissile bonds in the Factor VIII molecule: Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

REMARKS

Pending claims 28 to 85 have been cancelled and new claims 86 to 154 have been added in place thereof.

Support for new claim 117 is found on page 7, line 12 and on page 6, line 17 of the specification as originally filed. Support for new claim 128 in the description as originally filed is to be found on p. 10, lines 7-8 ("*diseases of thrombotic nature*"), and also on page 9, line 27 ("*such as thrombosis in particular*"). Support in the description as originally filed is to be found on page 7, line 12 to page 9, line 23. Support for new claims 141-143 in the application as originally filed is found in original claim 17 and claims dependent thereon. Support for new claims 144-150 in the application as originally filed for this new claim is found in original claim 26. Support for new claims 151-154 in the application as originally filed is found on page 3, line 4 and also on page 5, line 7, and in Example I (page 16, line 25 *et seq.*), and page 19, line 20 *et seq.*, *inter alia*.

It is submitted that no new matter is presented in the claims presented herewith. In view of the foregoing amendments and remarks, Applicants respectfully request favorable consideration and allowance of the present application.

Respectfully submitted,

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Dated: 1/28, 2003

WILDMAN, HARROLD, ALLEN & DIXON
225 West Wacker Drive
Chicago, Illinois 60606-1229
Telephone: (312) 201-2834
Facsimile: (312) 201-2327
e-mail: keefer@wildmanharrold.com

ATTACHMENT TO AMENDMENT OF SERIAL NO. 10/031,938
CONTAINING MARKED-UP CHANGES TO SPECIFICATION

On page 6, lines 17-32, continuing through to page 7, lines 1-10, please delete the existing paragraph and replace it with the following paragraph:

Hence, according to a third aspect, the present invention provides an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Advantageously, this inhibitor is characterized in that it comprises a protease inhibitor. Examples of protease inhibitors that can be used as anti-Factor VIII allo-antibody catalysed Factor VIII degradation inhibitors within the context of the present invention, without being limited thereto, are fluorophosphate-type inhibitors, such as DFP for example, or sulphonyl fluoride-type inhibitors, such as PMSF or AEBSF (4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (notably marked by Roche Diagnostics GmbH, Mannheim, Germany, under the trademark Pefabloc®)), for example. More particularly, this inhibitor is characterized in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and the Glu^{1794} – Asp^{1795} located within the A3 domain of the Factor VIII molecule. More preferably still, this inhibitor is characterized in that it comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro ;

a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; or

a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

On page 20, please delete the existing table and replace it with the following table:

Amino acid sequence	Cleavage site
Ser Val Ala Lys Lys His Pro (SVAKKHP)	$Arg^{372} - Ser^{373}$ $(R^{372} - S^{373})$
Asp Gln Arg Gln Gly Ala Glu (DQRQGAE)	$Glu^{1794} - Asp^{1795}$ $(E^{1794} - D^{1795})$
Asp Glu Asp Glu Asn Gln [Sr] Ser (DEDENQS)	$Tyr^{1680} - Asp^{1681}$ $(Y^{1680} - D^{1681}$

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents, Box PCT
United States Patent and Trademark Office
Washington, D.C. 20231
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/031,938	Srinivas Kaveri	TJK/209

INTERNATIONAL APPLICATION NO.	
PCT/EP00/06870	
I.A. FILING DATE	PRIORITY DATE
07/18/2000	07/21/1999

Timothy J Keefer
Wildman Harrold Allen & Dixon
225 West Wacker Drive
Chicago, IL 60606

CONFIRMATION NO. 8718

371 FORMALITIES LETTER

OC000000009308884

Date Mailed: 12/31/2002

NOTIFICATION OF DEFECTIVE RESPONSE

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as an Elected Office (37 CFR 1.495):

- U.S. Basic National Fee
- Priority Document
- Assignee Statement
- Biochemical Sequence Diskette
- Biochemical Sequence Listing
- Copy of IPE Report
- Copy of references cited in ISR
- Copy of the International Application
- Copy of the International Search Report
- Oath or Declaration
- Preliminary Amendments

Applicant's response filed 09/12/2002 is hereby acknowledged. The following requirements set forth in the NOTIFICATION of MISSING REQUIREMENTS mailed 04/03/2002 have not been completed.

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

Applicant is required to complete the response within a time limit of ONE MONTH from the date of this Notification or within the time remaining in the response set forth in the Notification of Missing Requirements, whichever is the longer. No extension of this time limit may be granted under 37 CFR 1.136, but the period for response set in the Notification of Missing Requirements may be extended under 37 CFR 1.136(a).

The following items **MUST** be furnished within the period set forth below:

- The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821-1.825 for the following reason(s):

[redacted]

- A copy of the "Sequence Listing" in computer readable form has been submitted. The content of the computer readable form, however, does not comply with the requirements of 37 CFR 1.822 and/or 1.832, as indicated on the attached marked-up copy of the "Raw Sequence Listing."
- The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).
- APPLICANT MUST PROVIDE:
  - An initial or substitute computer readable form (CRF) of the "Sequence Listing."
  - An initial or substitute paper copy or compact disc of the "Sequence Listing," as well as an amendment directing its entry into the specification.

- For questions regarding compliance to 37 CFR 1.821-1.825 requirements, please contact:
  - For Rules Interpretation, call (703) 308-4216
  - To Purchase PatentIn Software, call (703) 306-2600
  - For PatentIn Software Program Help, call (703) 306-4119 or e-mail at patin21help@uspto.gov or patin3help@uspto.gov

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

VONDA M WALLACE

Telephone: (703) 305-3736

PART 1 - ATTORNEY/APPLICANT COPY

U.S. APPLICATION NUMBER NO.	INTERNATIONAL APPLICATION NO.	ATTY. DOCKET NO.
10/031,938	PCT/EP00/06870	TJK/209

FORM PCT/DO/EO/916 (371 Formalities Notice)

TJK/209

IN THE UNITED STATES PATENT AND TRADEMARKS OFFICE

In re Application of: Kaveri	)	CATALYTIC ANTI-FACTOR VIII ANTIBODIES
Serial No.: 10/031,938	)	
Filed: 01/22/2002	)	
	)	Group Art Unit:

TRANSMITTAL LETTER

Commissioner of Patent and Trademarks
Washington, D.C. 20231

Sir:

Please find enclosed the following in the above referenced patent application:

1. Transmittal Letter (in duplicate);
2. Amendment of Sequence Listing ;
3. Paper copy of amended Sequence Listing;
4. Copy of Second Preliminary Amendment previously filed;
5. Two (2) 3.5 computer floppy disks containing the Sequence Listing in American Standard Code for Information Interchange (ASCII) text;
6. Duplicate Notice of Notification of Defective Response; and
7. Postcard.

Please acknowledge receipt of the above by returning the enclosed stamped, self-addressed receipt postcard.

Please charge any additional fees which include any extension fees due to **Deposit Account No. 23-2126**. A duplicate of this transmittal is enclosed.

Respectfully submitted,

Date: Jan 31, 2003

By: _____
Timothy J. Keefer, Reg. No. 35,567

Wildman, Harrold, Allen & Dixon
225 West Wacker Drive
Chicago, IL 60606
Ph. (312) 201-2000
Fax (312) 201-2555

TJK/209

IN THE UNITED STATES PATENT AND TRADEMARKS OFFICE

In re Application of: Kaveri	)	CATALYTIC ANTI-FACTOR VIII ANTIBODIES
Serial No.: 10/031,938	)	
Filed: 01/22/2002	)	
	)	Group Art Unit:

TRANSMITTAL LETTER

Commissioner of Patent and Trademarks
Washington, D.C. 20231

Sir:

Please find enclosed the following in the above referenced patent application:

1. Transmittal Letter (in duplicate);
2. Amendment of Sequence Listing ;
3. Paper copy of amended Sequence Listing;
4. Copy of Second Preliminary Amendment previously filed;
5. Two (2) 3.5 computer floppy disks containing the Sequence Listing in American Standard Code for Information Interchange (ASCII) text;
6. Duplicate Notice of Notification of Defective Response; and
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Please acknowledge receipt of the above by returning the enclosed stamped, self-addressed receipt postcard.

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Respectfully submitted,

Date: Jan 31, 2003

By: _____
Timothy J. Keefer, Reg. No. 35,567

Wildman, Harrold, Allen & Dixon
225 West Wacker Drive
Chicago, IL 60606
Ph. (312) 201-2000
Fax (312) 201-2555

[redacted]

#10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Kaveri et al.	)	CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES
Serial No.: 10/031,938	)	
Filed: January 22, 2002	)	Attorney Docket: TJK/209
	)	Group Art Unit:
	)	Examiner:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on 1/28, 2003.

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Date of Signature: 1/28, 2003

Assistant Commissioner for Patents
Washington, D.C. 20231

SECOND PRELIMINARY AMENDMENT

IN THE SPECIFICATION:

On page 6, lines 17-32, continuing through to page 7, lines 1-10, please delete the existing paragraph and replace it with the following paragraph:

Hence, according to a third aspect, the present invention provides an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Advantageously, this inhibitor is

characterized in that it comprises a protease inhibitor. Examples of protease inhibitors that can be used as anti-Factor VIII allo-antibody catalysed Factor VIII degradation inhibitors within the context of the present invention, without being limited thereto, are fluorophosphate-type inhibitors, such as DFP for example, or sulphonyl fluoride-type inhibitors, such as PMSF or AEBSF (4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (notably marked by Roche Diagnostics GmbH, Mannheim, Germany, under the trademark Pefabloc®)), for example. More particularly, this inhibitor is characterized in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and the $Glu^{1794} - Asp^{1795}$ located within the A3 domain of the Factor VIII molecule. More preferably still, this inhibitor is characterized in that it comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro ;

a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; or

a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

On page 20, please delete the existing table and replace it with the following table:

Amino acid sequence	Cleavage site
Ser Val Ala Lys Lys His Pro (SVAKKHP)	$Arg^{372} - Ser^{373}$ ($R^{372} - S^{373}$)
Asp Gln Arg Gln Gly Ala Glu (DQRQGAE)	$Glu^{1794} - Asp^{1795}$ ($E^{1794} - D^{1795}$)
Asp Glu Asp Glu Asn Gln Ser (DEDENQS)	$Tyr^{1680} - Asp^{1681}$ ($Y^{1680} - D^{1681}$

IN THE CLAIMS:

Please cancel pending claims 28 to 85.

Please enter new claims 86 to 154.

86. (New) A method of determining the presence of anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, which comprises :

i) isolating the plasma from a sample of blood taken from said mammal,

ii) isolating anti-Factor VIII allo-antibodies from said plasma ;

iii) placing said anti-Factor VIII allo-antibodies in contact with Factor VIII for a period of time sufficient to permit any degradation of said Factor VIII by said anti-Factor VIII allo-antibodies ; and

iv) determining, after said period of time, whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies.

87. (New) The method of claim 86, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated from said plasma by combining them with said Factor VIII.

88. (New) The method of claim 87, wherein said Factor VIII is coupled to a matrix.

89. (New) The method of claim 86, wherein in step ii), said anti-Factor VIII allo-antibodies are isolated by affinity chromatography.

90. (New) The method of claim 89, wherein in step ii), said affinity chromatography comprises the use of Factor VIII covalently coupled to a Sepharose matrix.

91. (New) The method of claim 90, wherein said Sepharose matrix is activated with cyanogen bromide.

92. (New) The method of claim 86, wherein in step iii), said Factor VIII is labelled with a labelling agent.

93. (New) The method of claim 92, wherein said labelling agent is a radio-labelling agent.

94. (New) The method of claim 93, wherein said radio-labelling agent is ^{125}I.

95. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of about 15 to about 40°C.

96. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of about 15 to about 40°C.

97. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, at a temperature of 38°C.

98. (New) The method of claim 86, wherein in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of about 10 hours, at a temperature of 38°C.

99. (New) The method of claim 86, wherein step iv) is carried out by a determination comprising a separation technique and a visualisation technique.

100. (New) The method of claim 99, wherein said separation technique is selected from the group consisting of gel electrophoresis, and gel filtration.

101. (New) The method of claim 100, wherein said gel electrophoresis is SDS PAGE.

102. (New) The method of claim 100, wherein said gel filtration is fast protein liquid chromatography gel filtration.

103. (New) The method of claim 100, wherein said visualisation technique is autoradiography.

104. (New) The method of claim 86, which further comprises :

v) characterising the site(s) in said Factor VIII molecule cleaved by said anti-Factor VIII allo-antibodies.

105. (New) The method of claim 104, wherein said characterisation is carried out by placing said Factor VIII in contact with said anti-Factor VIII allo-antibodies capable of degrading Factor VIII, separating and then sequencing the fragments of Factor VIII resulting therefrom.

106. (New) The method of claim 105, wherein said separation is carried out using a technique such as gel electrophoresis.

107. (New) The method of claim 106, wherein said separation is SDS PAGE.

108. (New) The method of claim 105, wherein said sequencing is carried out using a technique such as N-terminal sequencing.

109. (New) The method of claim 108, wherein said sequencing carried out using a technique such as N-terminal sequencing is by using an automatic protein microsequencer.

110. (New) The method of claim 104, wherein said sequencing locates scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

111. (New) An amino acid sequence :

Ser Val Ala Lys Lys His Pro .

112. (New) An amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

113. (New) An amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

114. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 111, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

115. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 112, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

116. (New) A peptide or non-peptide analogue of an amino acid sequence of claim 113, which is capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

117. (New) A method of neutralising catalytic anti-Factor VIII allo-antibodies comprising using an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

118. (New) The method of claim 117, wherein said inhibitor comprises a protease inhibitor.

119. (New) The method of claim 118, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

[redacted]

120. (New) The method of claim 117, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

121. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

122. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

123. (New) The method of claim 117, wherein said inhibitor comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

124. (New) A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of an anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

125. (New) The pharmaceutical composition of claim 124, wherein said anti-Factor VIII allo-antibody capable of degrading Factor VIII is as obtainable from the method of claim 86.

126. (New) A method of therapeutic treatment of a mammal suffering from a pathology resulting from abnormal level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one anti-Factor VIII allo-antibody capable of degrading Factor VIII, and a

pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier, is administered to said mammal.

127. (New) The method of claim 126, wherein said pathology results from the presence of an excess of Factor VIII in the blood thereof.

128. (New) The method of claim 127, wherein said pathology is of thrombotic nature.

129. (New) The method of claim 128, which is a therapeutic treatment of a mammal suffering from thrombosis.

130. (New) A pharmaceutical composition which comprises a pharmaceutically effective amount of a pharmaceutically active ingredient selected from the group consisting of a Factor VIII degradation inhibitor of claim 117, and a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable excipient, vehicle or carrier.

131. (New) A method of therapeutic treatment of a mammal suffering from a pathology resulting from the sub-physiological level of Factor VIII in the blood thereof, wherein a therapeutically effective amount of a pharmaceutically active ingredient selected from the group consisting of at least one Factor VIII degradation inhibitor, and a pharmaceutically acceptable salt thereof, is administered to said mammal.

132. (New) The method of claim 131, wherein said inhibitor comprises a protease inhibitor.

133. (New) The method of claim 132, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

134. (New) The method of claim 131, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

135. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

136. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

137. (New) The method of claim 131, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

138. (New) The method of claim 131, wherein said pathology is of haemophilic nature.

139. (New) The method of claim 138, wherein said pathology of haemophilic nature is a disease involving coagulation defects due to Factor VIII insufficiency.

140. (New) The method of claim 138, which is a method of therapeutic treatment of a mammal suffering from haemophilia A.

141. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro .

142. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence:

Asp Glu Asp Glu Asn Gln Ser .

143. (New) An anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

144. (New) A pharmaceutical composition, which comprises a pharmaceutically effective amount of an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

145. (New) The pharmaceutical composition of claim 144, which comprises a protease inhibitor.

146. (New) The pharmaceutical composition of claim 145, wherein said protease inhibitor is 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride.

147. (New) The pharmaceutical composition of claim 144, wherein said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

148. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro .

149. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser .

150. (New) The pharmaceutical composition of claim 144, which comprises a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

151. (New) An anti-Factor VIII allo-antibody, which has a catalytic activity capable of catalysing degradation of Factor VIII.

152. (New) An anti-Factor VIII allo-antibody which is obtainable by the method of claim 86.

153. (New) The anti-Factor VIII allo-antibody of claim 151, which cleaves the following scissile bonds in the Factor VIII molecule: Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

154. (New) The anti-Factor VIII allo-antibody of claim 152, which cleaves the following scissile bonds in the Factor VIII molecule: Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

REMARKS

Pending claims 28 to 85 have been cancelled and new claims 86 to 154 have been added in place thereof.

[redacted]

Support for new claim 117 is found on page 7, line 12 and on page 6, line 17 of the specification as originally filed. Support for new claim 128 in the description as originally filed is to be found on p. 10, lines 7-8 ("*diseases of thrombotic nature*"), and also on page 9, line 27 ("*such as thrombosis in particular*"). Support in the description as originally filed is to be found on page 7, line 12 to page 9, line 23. Support for new claims 141-143 in the application as originally filed is found in original claim 17 and claims dependent thereon. Support for new claims 144-150 in the application as originally filed for this new claim is found in original claim 26. Support for new claims 151-154 in the application as originally filed is found on page 3, line 4 and also on page 5, line 7, and in Example I (page 16, line 25 *et seq.*), and page 19, line 20 *et seq.*, *inter alia*.

It is submitted that no new matter is presented in the claims presented herewith. In view of the foregoing amendments and remarks, Applicants respectfully request favorable consideration and allowance of the present application.

Respectfully submitted,

Timothy J. Keefer
Attorney for Applicants
Reg. No. 35567

Dated: 1/28, 2003

WILDMAN, HARROLD, ALLEN & DIXON
225 West Wacker Drive
Chicago, Illinois 60606-1229
Telephone: (312) 201-2834
Facsimile: (312) 201-2327
e-mail: keefer@wildmanharrold.com

ATTACHMENT TO AMENDMENT OF SERIAL NO. 10/031,938
CONTAINING MARKED-UP CHANGES TO SPECIFICATION

On page 6, lines 17-32, continuing through to page 7, lines 1-10, please delete the existing paragraph and replace it with the following paragraph:

Hence, according to a third aspect, the present invention provides an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Advantageously, this inhibitor is characterized in that it comprises a protease inhibitor. Examples of protease inhibitors that can be used as anti-Factor VIII allo-antibody catalysed Factor VIII degradation inhibitors within the context of the present invention, without being limited thereto, are fluorophosphate-type inhibitors, such as DFP for example, or sulphonyl fluoride-type inhibitors, such as PMSF or AEBSF (4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (notably marked by Roche Diagnostics GmbH, Mannheim, Germany, under the trademark Pefabloc®)), for example. More particularly, this inhibitor is characterized in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and the Glu^{1794} – Asp^{1795} located within the A3 domain of the Factor VIII molecule. More preferably still, this inhibitor is characterized in that it comprises a peptide or non-peptide analogue of the amino acid sequence:

Ser Val Ala Lys Lys His Pro ;

a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; or

a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

On page 20, please delete the existing table and replace it with the following table:

Amino acid sequence	Cleavage site
Ser Val Ala Lys Lys His Pro (SVAKKHP)	$Arg^{372} - Ser^{373}$ $(R^{372} - S^{373})$
Asp Gln Arg Gln Gly Ala Glu (DQRQGAE)	$Glu^{1794} - Asp^{1795}$ $(E^{1794} - D^{1795})$
Asp Glu Asp Glu Asn Gln [Sr] Ser (DEDENQS)	$Tyr^{1680} - Asp^{1681}$ $(Y^{1680} - D^{1681}$

CATALYTIC ANTI-FACTOR VIII ALLO-ANTIBODIES.

FIELD OF THE INVENTION

The present invention relates to a method of determining the presence of catalytic anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, and of characterising the cleavage sites in said Factor VIII molecule by said catalytic anti-Factor VIII allo-antibodies.

The present invention also relates to an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

The present invention further relates to a pharmaceutical composition comprising said catalytic anti-Factor VIII allo-antibodies which are capable of degrading Factor VIII and which originate from said method of determination, and to a pharmaceutical composition comprising said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

Finally, the present invention relates to the application in therapeutics of said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor, of a pharmaceutical composition comprising said catalytic anti-Factor VIII allo-antibodies which are capable of degrading Factor VIII and which originate from said method of determination, and of a pharmaceutical composition comprising said anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor.

BACKGROUND TO THE INVENTION

Haemophilia A is an X chromosome-linked recessive disorder resulting in defective or deficient Factor VIII molecules, which, in its severe form, is a life-threatening and crippling haemorrhagic disease.

Infusion of homologous Factor VIII to patients with severe haemophilia A results, in 25% of the cases, in the emergence of anti-Factor VIII allo-antibodies (Ehrenforth, S., Kreuz, W., Scharrer, I., Linde, R., Funk, M., Güngör, T., Krackhardt, B. and Kornhuber, B., « *Incidence of development of factor VIII and factor IX inhibitors in haemophiliacs* », Lancet, 1992, *339*: 594-598), that inhibit Factor VIII procoagulant activity by steric hindrance of the interaction of Factor VIII either with stabilising molecules (Saenko, E. L., Shima, M., Rajalakshmi, K. J. and Scandella, D., « *A role for the C2 domain of factor VIII in binding to von Willebrand factor* », J. Biol. Chem., 1994, *269*: 11601-11605 ; and Saenko, E. L., Shima, M., Gilbert, G. E., and Scandella, D., « *Slowed release of thrombin-cleaved factor VIII from von Willebrand factor by a monoclonal and a human antibody is a novel mechanism for factor VIII inhibition* », J. Biol. Chem., 1996, *271*: 27424-27431), with molecules essential for its activity (Arai, M., Scandella, D., and Hoyer, L. W., « *Molecular basis of factor VIII inhibition by human antibodies : Antibodies that bind to the factor VIII light chain prevent the interaction of factor VIII with phospholipid* », J. Clin. Invest., 1989, *83*: 1978-1984 ; and Zhong, D., Saenko, E. L., Shima, M., Felch, M. and Scandella, D., « *Some human inhibitor antibodies interfere with factor VIII binding to Factor IX* », Blood, 1998, *92*: 136-142), or with activating molecules (Lubahn, B. C., Ware, J., Stafford, D. W., and Reiser, H. M., « *Identification of a FVIII epitope recognized by a human hemophilic inhibitor* », Blood, 1989, *73*: 497-499 ; and Neuenschwander, P. F., and Jesty, J., « *Thrombin-activated and factor Xa-activated human factor VIII : differences in cofactor activity and decay rate* », Arc. Biochem. Biophys., 1992, *296*: 426-434).

SUMMARY OF THE INVENTION

In an entirely surprising way, a discovery has been made by the Applicants of a degradation of Factor VIII by allo-antibodies of two high responder patients with severe haemophilia A, demonstrating a heretofore unknown mechanism

by which Factor VIII inhibitors may prevent the pro-coagulant function of Factor VIII.

The Applicant's discovery of catalytic anti-Factor VIII allo-antibodies is to the best of his knowledge the first report on the emergence of catalytic antibodies that are INDUCED upon treatment of patients with Factor VIII. It was heretofore considered very surprising, even absurd or unbelievable, that antibodies are formed, in the presence of Factor VIII, which would actually render the Factor VIII molecule inactive through catalytic hydrolysis (« proteolysis »). However, the catalytic antibodies reported so far, are all auto-antibodies found in the course of a disease process or in physiological conditions. Thus, induced antibodies are called ALLO-antibodies, the origin of which is clearly different from the origin of AUTO-antibodies in any auto-immune disease.

The calculated average Km and apparent Vmax for the reaction of anti-Factor VIII antibodies of one of the patients were 9.46 ± 5.62 μM and 85 ± 60 fmol.min^{-1}, respectively. The kinetic parameters of Factor VIII hydrolysis suggest a functional role for the catalytic immune response in the inactivation of Factor VIII *in vivo*.

The characterisation of anti-Factor VIII allo-antibodies as site-specific proteases hence provide new approaches to the treatment of diseases of a patient who possess anti-Factor VIII allo-antibodies.

Thus, according to a first aspect, the present invention provides a method of determining the presence of catalytic anti-Factor VIII allo-antibodies capable of degrading Factor VIII in a mammal, characterised in that it comprises :

i) isolating the plasma from a sample of blood taken from said mammal,

ii) isolating anti-Factor VIII allo-antibodies from said plasma ;

iii) placing said anti-Factor VIII allo-antibodies in contact with Factor VIII for a period of time sufficient to permit any degradation of said Factor VIII by said anti-Factor VIII allo-antibodies ; and

iv) determining, after said period of time, whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies.

According to an embodiment of step ii) of the method of the present invention, said anti-Factor VIII allo-antibodies are isolated from said plasma by combining them with said Factor VIII, said Factor VIII being preferably coupled to a matrix. Advantageously, in step ii), said anti-Factor VIII allo-antibodies are isolated by affinity chromatography. Preferably, in step ii), said affinity chromatography comprises the use of a Sepharose matrix, preferably activated with cyanogen bromide.

According to an embodiment of step iii) of the method of the present invention, said Factor VIII is labelled with a labelling agent, preferably a radio-labelling agent, such as ^{125}I in particular. Advantageously, in step iii), said Factor VIII is placed in contact with the anti-Factor VIII allo-antibodies for a period of time of between about 0.5 and about 30 hours, preferably about 10 hours, at a temperature of about 15 to about 40°C, preferably 38°C.

According to an embodiment of step iv) of the method of the present invention, the determination of whether said Factor VIII has effectively been degraded by said anti-Factor VIII allo-antibodies is carried out by a determination comprising a separation technique, such as gel electrophoresis, such as SDS PAGE in particular, or gel filtration, such as fast protein liquid chromatography gel filtration in particular, and a visualisation technique, such as autoradiography in particular.

In accordance with a further embodiment of the method of the present invention, said method is characterised in that it further comprises :

v) characterising the site(s) in said Factor VIII molecule cleaved by said anti-Factor VIII allo-antibodies.

According to an embodiment of step v) of the method of the present invention, said characterisation is carried out by placing said Factor VIII in contact with said anti-Factor VIII allo-antibodies capable of degrading Factor VIII, separating and then sequencing the fragments of Factor VIII resulting therefrom. Advantageously, said separation is carried out using a technique such as gel electrophoresis, such as SDS PAGE in particular, or gel filtration. Said sequencing is advantageously carried out using a technique such as N-terminal sequencing, such as by using an automatic protein microsequencer in particular. By using the said sequencing, the following scissile bonds are located : Arg^{372}-Ser^{373}, located between the A1 and A2 domains, Tyr^{1680}-Asp^{1681}, located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule.

According to a second aspect, therefore, the present invention provides an amino acid sequence :

Ser Val Ala Lys Lys His Pro ;

an amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; and

an amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

The present invention also extends to variants or analogues of this or any other sequence of Factor VIII which are capable of inhibiting any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody. Within the context of the present invention, such a variant can be, for example, a peptide or non-peptide analogue of an amino acid sequence described *supra* which inhibits any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody. Such a variant can be, for example, a variant of the sequence which is either shorter by a few amino acids, at the N-terminal, the C-terminal, or both termini, for example, or longer by a few amino acids (it being possible to obtain such variants by chemical synthesis or by enzymatic digestion of the naturally occurring molecule), so long as the variant inhibits any site in the Factor VIII molecule which is susceptible to being lysed by an anti-Factor VIII allo-antibody.

Hence, according to a third aspect, the present invention provides an anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor. Advantageously, this inhibitor is characterised in that it comprises a protease inhibitor. Examples of protease inhibitors that can be used as anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitors within the context of the present invention, without being limited thereto, are fluorophosphate-type inhibitors, such as DFP for example, or sulphonyl fluoride-type inhibitors, such as PMSF or AEBSF (4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride (notably marketed by Roche Diagnostics GmbH, Mannheim, Germany, under the trademark Pefabloc®)), for example. More particularly, this inhibitor is characterised in that said inhibitor inhibits cleavage of the scissile bonds : Arg^{372}-Ser^{373}, located between the A1 domains, Tyr^{1680}-Asp^{1681} , located on the N-terminus of the A3 domain, and Glu^{1794}-Asp^{1795} located within the A3 domain of the Factor VIII molecule. More preferably still, this inhibitor is characterised in that it comprises a peptide or non-peptide analogue of the amino acid sequence :

Ser Val Ala Lys Lys His Pro ;

a peptide or non-peptide analogue of the amino acid sequence :

Asp Glu Asp Glu Asn Gln Ser ; or

a peptide or non-peptide analogue of the amino acid sequence :

Asp Gln Arg Gln Gly Ala Glu .

The Factor VIII degradation inhibitors as defined *supra*, as well as their addition salts, in particular their pharmaceutically acceptable addition salts, have a very valuable pharmacological profile in that they possess neutralising activity towards anti-Factor VIII allo-antibodies.

These properties justify their application in therapeutics and the invention further relates, by way of drugs, to the Factor VIII degradation inhibitors above, as well as their addition salts, in particular their pharmaceutically acceptable addition salts.

They will therefore be particularly indicated in the treatment of diseases of, *inter alia*, haemophilic nature, more particularly diseases involving coagulation defects due to Factor VIII insufficiency.

An example of their use which may be mentioned is the treatment of high responder patients with diseases such as mild or severe haemophilia A, for example (in the case in which catalytic antibodies are found in these patients), on the one hand, and/or, on the other hand, patients suffering from auto-immune diseases for example (in the case in which catalytic antibodies are found in these patients).

Thus, according to a fourth principal aspect, the present invention provides a solution to a long-felt need through a pharmaceutical composition characterised in that it comprises a pharmaceutically effective amount of at least one anti-Factor VIII allo-antibody capable of degrading Factor VIII, as defined *supra*, notably as obtainable from the method described *supra*, or one of its pharmaceutically acceptable addition salts incorporated in a pharmaceutically acceptable excipient, vehicle or carrier.

Further, according to a fifth principal aspect, the present invention provides a pharmaceutical composition characterised in that it comprises a pharmaceutically effective amount of at least one Factor VIII degradation inhibitor, as defined *supra*, or one of its pharmaceutically acceptable addition salts incorporated in a pharmaceutically acceptable excipient, vehicle or carrier.

These compositions can be administered by the buccal, rectal, parenteral, transdermal, ocular, nasal or auricular route, for example.

These compositions can be solid or liquid and can be presented in the pharmaceutical forms commonly used in human medicine, such as, for example, simple or coated tablets, gelatine capsules, granules, suppositories, injectable preparations, transdermal systems, eye drops, aerosols and sprays, and ear drops. They are prepared by the customary methods. The active principle, which consists of a pharmaceutically effective amount of at least one Factor VIII degradation inhibitor as defined *supra*, or one of its pharmaceutically acceptable addition salts can be incorporated therein together with excipients normally employed in pharmaceutical compositions, such as talc, gum Arabic, lactose, starch, magnesium stearate, polyvidone, cellulose derivatives, cocoa butter, semi-synthetic glycerides, aqueous or non-aqueous vehicles, fats of animal or vegetable origin, glycols, various wetting agents, dispersants or emulsifiers, silicone gels, certain polymers or

copolymers, preservatives, flavourings and colours. The preferred pharmaceutical form is an injectable form.

The invention also covers a pharmaceutical composition with neutralising activity which can be used especially as a favourable treatment of diseases such as haemophilia A with production of anti-Factor VIII allo-antibodies ; autoimmune diseases with anti-Factor VIII allo-antibodies (in case catalytic antibodies are found in these patients) in particular, said composition being characterised in that it comprises a pharmaceutically effective amount of at least one Factor VIII degradation inhibitor above, or one of its pharmaceutically acceptable addition salts incorporated in a pharmaceutically acceptable excipient, vehicle or carrier.

The invention also covers a method of therapeutic treatment of a mammal suffering from a pathology resulting from the level of Factor VIII in the blood thereof, characterised in that a therapeutically effective amount of at least one Factor VIII degradation inhibitor as defined *supra* or one of its pharmaceutically acceptable addition salts is administered to the said mammal.

This method affords especially a favourable treatment of diseases of haemophilic nature, in particular a pathology resulting from a lack of Factor VIII in the blood thereof.

The invention also covers a pharmaceutical composition with anti-thrombotic activity which can be used especially as a favourable treatment of diseases such as thrombosis in particular, said composition being characterised in that it comprises a pharmaceutically effective amount of at least one anti-Factor VIII allo-antibody capable of degrading Factor VIII, notably as obtainable from the method described above, or one of its pharmaceutically acceptable addition salts incorporated in a pharmaceutically acceptable excipient, vehicle or carrier.

The invention also covers a method of therapeutic treatment of mammals, characterised in that a therapeutically effective amount of at least one anti-Factor VIII allo-antibody as defined *supra* or one of its pharmaceutically acceptable addition salts is administered to the said mammal.

This method affords especially a favourable treatment of diseases of thrombotic nature, in particular said pathology resulting from the presence of an excess of Factor VIII in the blood thereof.

In human and animal therapeutics, the anti-Factor VIII allo-antibodies or the Factor VIII degradation inhibitors as defined *supra* can be administered by themselves or in association with a physiologically acceptable excipient, in any form, in particular orally in the form of gelatine capsules or tablets, or parenterally in the form of injectable solutions. It is possible to envisage other forms of administration such as suppositories, ointments, creams, gels or aerosol preparations.

Within the context of the present invention, the following terms are used :

« catalytic anti-Factor VIII allo-antibodies », which is understood as meaning antibodies directed to Factor VIII endowed with a catalytic activity induced in haemophilia A patients upon transfusion with therapeutic preparations of Factor VIII ;

« Factor VIII », which is understood as meaning a co-enzyme of Factor IX in the enzymatic cleavage of Factor X during the blood coagulation process ;

« degradation of Factor VIII », which is understood as meaning the generation of fragments from Factor VIII that do not appear due to a spontaneous hydrolysis, or due to hydrolysis by physiologically cleaving

enzymes, *i.e.* thrombin, activated Factor IX, activated Factor X, and activated protein C ;

« anti-Factor VIII allo-antibody-catalysed Factor VIII degradation inhibitor », which is understood as meaning any peptide, belonging or not to the Factor VIII sequence, or protease inhibitor that are capable of specifically neutralising the hydrolysing activity of anti-Factor VIII catalytic antibodies ;

DESCRIPTON OF A PREFERRED EMBODIMENT OF THE PRESENT INVENTION

Human recombinant Factor VIII was radio-labelled with ^{125}I. Anti-Factor VIII allo-antibodies were affinity-purified from the plasma of three haemophilia patients with inhibitor on a Sepharose matrix to which immunopurified human Factor VIII had been coupled. Affinity-purified anti-Factor VIII antibodies of patients Bor, Che and Wal inhibited Factor VIII pro-coagulant activity up to 57.0, 64.0 and 43.0 BU/mg of IgG, respectively.

Co-incubation of labelled Factor VIII with the anti-Factor VIII allo-antibodies resulted, in the case of two patients out of three, in the proteolysis of the molecule. The specificity of the hydrolysis on the antibody combining sites of anti-Factor VIII allo-antibodies of the IgG isotype was demonstrated. Co-incubation of [^{125}I]-Factor VIII with affinity-purified anti-Factor VIII IgG of patients Bor and Wal in the presence of the protease inhibitors aprotinin (0.15 µM), E-64 (28 µM), EDTA (1.3 µM), leupeptin (10 µM), and pepstatin (10 µM) did not result in inhibition of proteolytic activity.

The Applicants have characterised the major cleavage sites for catalytic IgG in the Factor VIII molecule, to be as follows: Arg^{372} - Ser^{373}, located between the A1 and A2 domains of Factor VIII ; Tyr^{1680} - Asp^{1681}, located on the

N-terminus of the A3 domain ; and Glu^{1794} - Asp^{1795} located within the A3 domain.

The time and dose-dependency of the hydrolysis of Factor VIII by anti-Factor VIII allo-antibodies has been demonstrated. In particular, hydrolysis was observed under conditions where anti-Factor VIII IgG and Factor VIII were co-incubated at molar ratios that were 80- to 9500-fold lower than those expected to be present in patients' plasma, suggesting that hydrolysis is a mechanism of Factor VIII inactivation by the patients' allo-antibodies *in vivo*.

The Applicants have further investigated the kinetics of antibody-mediated hydrolysis of Factor VIII by incubating anti-Factor VIII IgG of patient Wal with increasing concentrations of unlabeled Factor VIII in the presence of a fixed concentration of [^{125}I]-Factor VIII. The curves of the reciprocal of the velocity plotted as a function of the reciprocal of the substrate concentration were linear (r=0.99), suggesting that the reaction conformed to simple Michaelis-Menten kinetics, as already observed for polyclonal catalytic auto-antibodies. The apparent catalytic efficiency, Vmax and rate of hydrolysis of anti-Factor VIII allo-antibodies were calculated in the case of patient Wal. The kinetic parameters of hydrolysis calculated *in vitro*, suggest that proteolysis may be a mechanism of Factor VIII inactivation by patients' allo-antibodies *in vivo*.

The association of Factor VIII with von Willebrand Factor (vWF) increases the catalytic rate of thrombin for Factor VIII, whereas it protects Factor VIII from hydrolysis by activated protein C (APC). The addition of vWF to Factor VIII resulted in partial inhibition of hydrolysis of Factor VIII by anti-Factor VIII IgG, *i.e.* 36.9%, when purified vWF and Factor VIII were mixed using a wt/wt ratio similar to that present in normal plasma, *i.e.* 30 µg/ml of vWF versus 300 ng/ml of Factor VIII.

The identification of anti-Factor VIII allo-antibodies as catalytic antibodies extends the spectrum of catalytic immune responses, in addition to previous reports of hydrolysing antibodies against vasoactive intestinal peptide (VIP) in asthma patients, DNA-hydrolysing antibodies in patients with SLE and thyroglobulin-specific catalytic antibodies in patients with autoimmune thyroiditis. This is also the first report to the knowledge of the Applicants of the induction of a catalytic antibody in the human, in response to exogeneous administration of a protein antigen. The kinetic parameters of Factor VIII hydrolysis by anti-Factor VIII IgG exhibiting catalytic properties and the estimated amounts of these antibodies in plasma, suggest a functional role for the catalytic immune response in inactivating Factor VIII *in vivo*. Within a polyclonal mixture of anti-Factor VIII allo-antibodies which differ in their functional properties, catalytic antibodies may inhibit Factor VIII pro-coagulant activity at faster rates than non-catalysing anti-Factor VIII antibodies. Identification of peptide epitopes that are the targets for proteolytic anti-Factor VIII antibodies may thus be critical for our understanding of the pathophysiology of the Factor VIII inhibitor response. Furthermore, the characterisation of Factor VIII inhibitors as site-specific proteases will provide new approaches to the treatment of patients possessing anti-Factor VIII allo-antibodies.

BRIEF DESCRIPTION OF THE FIGURES

The invention will be better understood and other objects, characteristics and advantages thereof will become more clearly apparent from the following explanatory description referring to the attached Figures, which are given solely by way of non-limiting Examples illustrating the specificity of the cleavage of Factor VIII by anti-Factor VIII allo-antibodies.

Figure 1: Hydrolysis of [^{125}I]-Factor VIII by affinity-purified anti-Factor VIII IgG antibodies of haemophilia A patients with inhibitor

Figure 1(A):

[125I]-labelled Factor VIII was incubated with affinity-purified anti-Factor VIII IgG of patients Bor (lane Bor), Che (lane Che) and Wal (lane Wal), or with buffer alone (lane 1) for 10 h at 38°C prior to SDS-PAGE and autoradiography. In two of the three patients (Bor and Wal), incubation of Factor VIII with affinity-purified anti-Factor VIII IgG resulted in hydrolysis of the Factor VIII molecule. In contrast, the migration profile of Factor VIII was unchanged when [^{125}I]-labelled Factor VIII was incubated with anti-Factor VIII IgG purified from the plasma of patient Che (lane Che). The migration profile of Factor VIII was also unchanged upon incubation with human monoclonal M061 anti-digoxin IgG (mAb) or with normal unfractionated polyclonal human IgG (Sandoglobulin® , IVIg) that exhibit no inhibitory activity to Factor VIII.

Figure 1(B):

Flow-throughs of the affinity columns were devoid of anti-Factor VIII antibodies as determined by ELISA, and did not hydrolyse [^{125}I]-labelled Factor VIII.

Figure 1(C):

Removal of IgG from the acid eluates containing affinity-purified anti-Factor VIII antibodies of patients Wal and Bor by chromatography on protein G, resulted in the loss of their hydrolytic activity to Factor VIII.

Figure 2: Size exclusion chromatography of the catalytic activity of anti-Factor VIII antibodies

Figure 2(A):

To further exclude the possibility that the proteolytic activity of the antibodies was due to contaminating proteases, affinity-purified anti-Factor VIII antibodies of patient Wal were treated with 8 M urea and subjected to size exclusion chromatography. A major peak was isolated in fraction 25 that corresponded to IgG as indicated by ELISA. The hydrolysing activity co-eluted with the IgG fraction and that the activity was not detected in fractions in which IgG was not present (*e.g.*, fraction 35).

Figure 2(B):

The major peak that was isolated in fraction 25 corresponded to IgG as indicated by SDS-PAGE of the radio-labelled content of the fraction.

Figure 3: Dose- and time-dependency of proteolysis of [^{125}I]-Factor VIII by affinity-purified anti-Factor VIII antibodies of haemophilia A patients with inhibitor.

The kinetics of the hydrolysis of Factor VIII by anti-Factor VIII allo-antibodies of patients Bor and Wal. The rate of hydrolysis of [^{125}I]-labelled Factor VIII by anti-Factor VIII IgG of patient Wal was faster than that exhibited by anti-Factor VIII IgG of patient Bor, suggesting either that catalytic antibodies of the patients exhibit different kinetic properties, or, alternatively, that the proportion of catalytic antibodies among the anti-Factor VIII antibodies differ between the patients.

Figure 4: Hydrolysis of [^{125}I]-Factor VIII by anti-Factor VIII IgG antibodies in the presence of increasing amounts of cold Factor VIII

Kinetics of antibody-mediated hydrolysis of Factor VIII by incubating anti-Factor VIII IgG of patient Wal with increasing concentrations of unlabelled Factor VIII in the presence of a fixed concentration of [^{125}I]-Factor VIII. The addition of increasing amounts of unlabelled Factor VIII resulted in dose-dependent inhibition of hydrolysis of [^{125}I]-Factor VIII by anti-Factor VIII IgG. Saturation of Factor VIII hydrolysis was not attained with the maximum concentration of Factor VIII that was used (*i.e.* 1.7 μM). The curves of the reciprocal of the velocity plotted as a function of the reciprocal of the substrate concentration were linear (r=0.99), suggesting that the reaction conformed to simple Michaelis-Menten kinetics, as already observed for polyclonal catalytic auto-antibodies.

Figure 5: Inhibition of catalytic activity of anti-Factor VIII IgG of patient Wal

The proteolysis of radio-labelled Factor VIII by the anti-Factor VIII allo-antibodies of patient Wal was inhibited to about 62% when the antibodies and Factor VIII were co-incubated in the presence of Pefabloc® (marketed by Roche Diagnostics GmbH, Mannheim, Germany), indicating the potency of certain serine protease inhibitor to neutralise the catalytic activity of some of the catalytic antibodies.

EXAMPLES

Example I: Affinity-purification of anti-Factor VIII antibodies

Antibodies were isolated from plasma by ammonium sulphate precipitation. Antibodies reactive with Factor VIII were then affinity-purified on a CNBr-activated Sepharose 4B matrix to which immuno-purified commercial human plasma-derived Factor VIII had been coupled (25000 U/3 g of gel). The

flow-throughs of the columns were collected. After extensive washing with PBS pH 7.4, anti-Factor VIII antibodies were eluted using 0.2 M glycine pH 2.8, dialysed against PBS and concentrated with Centriprep. Flow-throughs and eluates were aliquoted and stored at -20'C until use. $F(ab')_2$ fragments of anti-Factor VIII antibodies were prepared as previously described.

The concentration of anti-Factor VIII IgG was 130, 20 and 280 µg per 10 mg of IgG applied to the column in the case of patients Bor, Che and Wal, respectively, (*i.e.*, 143 ± 130 µg/ml of unfractionated plasma), which is in agreement with previous observations.

Example II: Factor VIII-neutralising activity

The Factor VIII-neutralising activity of anti-Factor VIII antibodies was determined by the method of Kasper *et al.* and expressed as Bethesda units (BU) (ref). BU were defined as the inverse of the concentration of IgG which causes 50% inhibition of Factor VIII procoagulant activity. Residual Factor VIII activity was measured in a one-stage assay by determination of the activated partial thromboplastin time using human plasma depleted of Factor VIII (Behring) as substrate and human placental pathromtin® (Behring) as activators. Heated plasma or immunopurified anti-Factor VIII IgG to be tested, were incubated with pooled citrated human plasma for 2 h at 37°C. The clotting time of four serial dilutions of a reference plasma pool (Immuno AG, Wien) was compared with the clotting time of three dilutions of each sample to be tested. Dilutions were carried out in Owren-Koller buffer (Diagnostica Stago). Inter-assay variation ranged between 1 and 2.5%.

Affinity-purified anti-Factor VIII antibodies of patients Bor, Che and Wal inhibited Factor VIII pro-coagulant activity up to 57.0, 64.0 and 43.0 BU/mg of IgG, respectively.

Example III: Assay for hydrolysis of Factor VIII

Commercial human recombinant Factor VIII was labelled with ^{125}I to a specific activity of 11.6 nCi/µg, by using the iodogen method. [^{125}I]-Factor VIII (1.5 to 150 ng) was incubated in 50 µl of 50 mM tris-HCl pH 7.7, 100 mM glycine, 0.025% Tween-20 and 0.02% NaN_3 alone or with 17 to 1667 nM of immuno-purified anti-Factor VIII IgG for 5 min to 10 hours at 38°C. Human monoclonal anti-digoxin IgG M061 (mAb) and normal unfractionated human polyclonal IgG (IVIg, Sandoglobulin®), were used as negative controls. Samples were mixed 1:1 with Laemmli's buffer without mercaptoethanol, and were subjected to SDS electrophoresis without boiling, after loading 20 µl of each sample per lane. Samples were run in parallel on 7.5% and 15% SDS-PAGE under non-reducing conditions, after loading 20 µl of each sample per lane. Migration was performed at room temperature using a mini-PROTEAN II system at 25 mA/gel, until the dye front reached the bottom of the gel. The gels were then dried and protein bands revealed using X-OMAT AR. Following autoradiography, the Factor VIII bands of apparent molecular weight 200 and 300 kDa that are consistently hydrolysed by anti-Factor VIII IgG, were scanned so as to allow for the calculation of the rate of hydrolysis of labelled Factor VIII.

Example IV: Fast protein liquid chromatography gel filration

A hundred µl aliquot of anti-Factor VIII IgG of patient Wal (740 µg) treated with 8M urea was subjected to gel filtration on a superose-12 column equilibrated with PBS-0.01% azide at a flow rate of 0.2 ml/min. Five hundred µl fractions were collected and assayed for the presence of IgG by sandwich ELISA and for Factor VIII proteolytic activity after ten-fold dilution. The proteins in fraction 25 were radiolabelled with ^{125}I and subjected to SDS-PAGE under non-reducing conditions in parallel with normal polyclonal human IgG. The gel was stained with Comassie Blue, and also autoradiographed ; both images were then overlaid. A major peak was

isolated in fraction 25 that corresponded to IgG as indicated by ELISA and SDS-PAGE of the radiolabelled content of the fraction. The hydrolysing activity co-eluted with the IgG fraction and that the activity was not detected in fractions in which IgG was not present (*e.g.*, fraction 35).

Example V: Analysis of NH_2-terminal sequences

Unlabelled human recombinant Factor VIII sucrose formulation (rDNA-BHK) (300 µg, octocog alfa, Bayer Corporation, Berkeley, CA) was treated with the anti-Factor VIII IgG of patient Wal (74 µg) in 1500 µl of 50 mM tris-HCl pH 7.7, 100 mM glycine, 0.025% tween-20 and 0.02% NaN_3 for 24 hours at 38°C. The resultant Factor VIII fragments were run on a 10% SDS-PAGE at 50 mA under non-reducing conditions and transferred for 2 hours at 100 mA on a Hybond-P PVDF membrane (Amersham, Little Chalfont, England) in 10 mM CAPS, 10% ethanol at pH 11.0. After staining with coomassie blue, visible bands were cut and subjected to N-terminal sequencing, using an automatic protein microsequencer Prosize 492 cLC (PE-Applied Biosystems, Foster City, CA). The amount of protein sequenced ranged from 0.5 to 2 pmoles, depending on the fragment.

The major scissile bonds were as follows: Arg^{372} - Ser^{373} (R^{372} - S^{373}), located between the A1 and A2 domains of Factor VIII ; Tyr^{1680} - Asp^{1681} (Y^{1680} -D^{1681}), located in the N-terminus of the A3 domain ; and Glu^{1794} - Asp^{1795} (E^{1794}-D^{1795}) located within the A3 domain. Multiple site cleavage of Factor VIII by anti-Factor VIII antibodies might originate from individual antibodies with polyspecific catalytic activities or polyclonal populations of antibodies, each exhibiting a unique cleavage site specificity.

Amino acid sequence	Cleavage site
Ser Val Ala Lys Lys His Pro (SVAKKHP)	Arg^{372} - Ser^{373} (R^{372} - S^{373})
Asp Gln Arg Gln Gly Ala Glu (DQRQGAE)	Glu^{1794} - Asp^{1795} (E^{1794} - D^{1795})
Asp Glu Asp Glu Asn Gln Sr (DEDENQS)	Tyr^{1680} - Asp^{1681} (Y^{1680} - D^{1681})

Example VI: Inhibition studies were performed using Pefabloc®, a generic inhibitor of serine proteases

Hydrolysis of [^{125}I]-Factor VIII by affinity-purified anti-Factor VIII IgG antibodies of haemophilia A patients with inhibitor in the presence of Pefabloc®. [^{125}I]-Factor VIII (150 ng) was incubated alone, with 50 µg/ml of immunopurified anti-Factor VIII IgG of patient Wal or in the presence of both anti-Factor VIII IgG and 4 mM of the serine protease inhibitor Pefabloc® (Boehringer) for 5 h at 38°C. Factor VIII was then analysed by 7.5% SDS-PAGE under non-reducing conditions. Following autoradiography, the Factor VIII bands of apparent molecular weight 200 and 300 kDa that are consistently hydrolysed by anti-FVIII IgG, were scanned so as to allow for the calculation of the % of hydrolysis of labelled Factor VIII.

The proteolysis of radiolabelled Factor VIII by the anti-Factor VIII allo-antibodies of patient Wal was inhibited to about 62% when the antibodies and Factor VIII were co-incubated in the presence of Pefabloc®, indicating the potency of some serine protease inhibitor to neutralise the catalytic activity of some catalytic antibodies.

Further Observation :

Upon screening the purified IgG of TEN high responder patients with haemophilia A using ^{125}I-radiolabelled Factor VIII as the target molecule, a change was observed in the migration profile of Factor VIII in the case of six patients. These results substantiate the Applicant's previous observations and indicate that catalytic anti-Factor VIII antibodies are found in about 60% of the patients.